

Chemical composition, microbiological properties, and fatty acid profile of Italian-type salami with pork backfat substituted by emulsified canola oil

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ABSTRACT: *Vegetable oils have been used to substitute pork backfat to improve the fatty acid profile of fermented sausages. The aim of this study was to assess the chemical composition, microbiological properties, and fatty acid profile of Italian-type salami with pork backfat substituted (15% and 30%) by emulsified canola oil. Fat contents decreased while moisture contents increased in Italian-type salami with emulsified canola oil. The growth of lactic acid bacteria in salami was not affected by canola oil and absence of fecal coliforms, coagulase-positive staphylococci, and Salmonella were reported during processing of fermented sausages. Lower levels of saturated fatty acids (SAFAs), higher levels of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) were observed in salami with emulsified canola oil. Together, our results indicated that substituting pork backfat with emulsified canola oil improved the nutritional values of Italian-type salami.*

Key words: *fermented sausages, vegetable oils, lipid content, polyunsaturated fatty acids, nutritional value.*

Composição química, propriedades microbiológicas e perfil de ácidos graxos de Salame tipo Italiano com substituição da gordura animal por óleo de canola emulsionado

RESUMO: *O uso de óleos vegetais como substitutos da gordura animal em embutidos fermentados tem surgido como uma alternativa de produzir produtos cárneos com um melhor perfil lipídico. Assim, o objetivo deste trabalho foi avaliar a composição química, propriedades microbiológicas e o perfil de ácidos graxos de salame tipo Italiano elaborado com a substituição parcial da gordura suína (15 e 30%) por emulsão com óleo de canola. A incorporação do óleo de canola reduziu o teor de gordura e aumentou o teor de umidade dos embutidos fermentados. Em relação às características microbiológicas, a adição de óleo de canola emulsionado não alterou o crescimento das bactérias lácticas durante o processamento. Ainda, não foram detectadas contagens de coliformes fecais, staphylococci coagulase positiva e Salmonella. Em relação ao perfil de ácidos graxos, foi possível observar a redução nos teores totais de ácidos graxos saturados (SAFAs) e uma elevação nos valores de ácidos graxos monoinsaturados (MUFAs) e poliinsaturados (PUFAs) nos tratamentos adicionados de óleo de canola. Dessa forma, a substituição da gordura suína por óleo de canola emulsionado em salame tipo Italiano torna-se uma alternativa interessante na produção de produtos cárneos com melhores características nutricionais.*

Palavras-chave: *embutido curado fermentado, óleo vegetal, conteúdo lipídico, ácidos graxos poliinsaturados, valor nutricional.*

INTRODUCTION

Currently, the relation between fat intake and health is well known. Consumers are constantly seeking foods with a lower content of fat and cholesterol and with a favorable fatty acid profile (OSPINA et al., 2012). Although, meat and meat products are excellent sources of nutrients, they are rich in saturated fats and cholesterol and both are associated with higher risks of cancer, heart diseases, and obesity (ARIHARA, 2006). Therefore, reformulation is an approach to develop meat products with better nutritional values for consumers (SINGH et al., 2014).

The fatty acid profile of meat and meat products can be changed by supplementing animal

feeds with monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) or by adding different lipids into meat products during processing (ARIHARA, 2006; FRUET et al., 2016). Foods with higher amounts of MUFAs and PUFAs are recommended by health professionals because they are associated with lower risks of cancer of the breast, colon, coronary, and brain diseases (NETTLETON et al., 2016).

Vegetable oils including olive, canola, soybean, sunflower, and corn have been used to substitute animal fats in cooked (CHOI et al., 2010; YUNES et al., 2013; ASUMING-BEDIAKO et al., 2014), fresh (SELANI et al., 2016), and fermented meat products (PELSE et al., 2007; DEL NOBILE et al., 2009; MUGUERZA et al., 2011) to improve

their nutritional values. Addition of emulsified canola oil in Italian-type salami did not affect its physico-chemical characteristics (pH, a_w , weight loss, color and lipid oxidation) and sensory attributes (BACKES et al., 2013). Because of a favorable lipid profile [low SAFAs, high MUFAs and PUFAs, and rich in linolenic fatty acid (C18:3n3)], canola oil is used to reformulate meat products replacing pork backfat (BAEK et al., 2016). Moreover, vegetable oils can improve the PUFA/SAFA ratio that is used to evaluate the nutritional quality of meat products.

The aim of this study was to evaluate the chemical composition, microbiological properties, and fatty acid profile of Italian-type salami with partial substitution of pork fat (15% and 30%) by emulsified canola oil.

MATERIALS AND METHODS

To improve the stability of canola oil in fermented meat products, a soy protein isolate-stabilized oil/water emulsion was made. The emulsion was prepared as previously described by BLOUKAS et al. (1997). Briefly, four parts of water (5°C) were mixed with one part of isolated soy protein (Bremil Food Industry, Arroio do Meio, Brazil) and followed by slow addition of six parts of canola oil (Bunge, São Paulo, Brazil). Formulation of fermented meat products consisted of 62.09% pork meat, 19.10% beef, 2.91% salt (Synth, Diadema, Brazil), 0.28% glucose (Synth, Diadema, Brazil), 0.28% sodium nitrite and nitrate (Bremil Food Industry, Arroio do Meio, Brazil), 0.23% sodium ascorbate (Bremil Food Industry, Arroio do Meio, Brazil), 0.19% sucrose (Synth, Diadema, Brazil), 0.19% pepper (Kitano, São Paulo, Brazil), 0.19% garlic powder (Knorr, São Paulo, Brazil), 0.19% nutmeg (MasterFoods, São Paulo, Brazil), and 0.02% starter culture containing *Pediococcus pentosaceus* and *Staphylococcus xylosus* (Bactoform SPX, Christian Hansen, Copenhagen, Denmark). Three different formulations were prepared: Control (C), 14.33% pork backfat; Treatment 1 (T1), 12.18% pork backfat and 2.15% emulsified canola oil; and treatment 2 (T2), 10.03% pork backfat and 4.30% emulsified canola oil. The addition of emulsified canola oil in T1 and T2 corresponded to a reduction of 15% and 30% of pork backfat, respectively. These levels of replacement pork backfat by emulsified canola oil were chosen because are considered intermediated. In fermented meat sausages, it is notable the presence of pork backfat and higher levels of replacement may produce rejection by consumers. The ingredients were mixed in a mixing machine (in batches of 5kg) (Menoncin, Erechim, Brazil) and manually stuffed into

artificial collagen casings (60mm×15cm) (Viscofan, Naturin, Weinheim, Germany). Sausages were then placed in a solution containing 20% potassium sorbate and allowed to ripen in a laboratory cabinet (Menoncin, Erechim, Brazil) for 28 days until water activity of 0.87 was attained. Temperature and relative humidity (RH) were programmed as follows: day 1, 25°C/95%; day 2, 24°C/93%; day 3, 23°C/90%; day 4, 22°C/85%; day 5, 21°C/80%; day 6, 20°C/75%; and days 7-28, 18°C/75%.

Moisture, fat, protein, and ash contents of salami were determined according to the AOAC (2005) methods. Moisture content was determined in an oven at 105°C until constant weight was attained. Total fat was extracted following the method as previously described by BLIGH & DYER (1959). Protein content was measured using the Kjeldahl method with digestion, distillation, and titration steps. Ash content was determined by heating at 500°C.

Microbiological properties of the salami were evaluated on days 0, 5, 7, 14, 21, and 28. Overall, 25g of samples were homogenized in 225ml of 0.1% peptone water (Oxoid Unipath Ltd., Basingstoke, Hampshire, UK), and serial dilutions were used for the microbiological analysis. Lactic acid bacteria (LAB), total coliforms, fecal coliforms, coagulase-positive staphylococci, and *Salmonella* were determined as described by DOWNES & ITO (2000).

After extraction, total lipids were methylated using 12% boron trifluoride-methanol solution (w/w), as reported by JOSEPH & ACKMAN (1992). Fatty acids methyl esters (FAMES) were measured using a gas chromatograph (3300, Varian, Sunnyvale, USA) equipped with a flame ionization detector. Separation of FAMES was performed on a Carbowax column (30m×0.25mm ID×0.25µm film thickness) (J & W Scientific, Santa Clara, USA). During chromatographic analysis, oven temperature was raised from 140°C to 220°C at 2.5°C min⁻¹, and temperatures of the injector and detector were set at 230°C and 250°C, respectively. Helium was used as a carrier gas with a flow rate of 1.6mL min⁻¹. Identification of FAMES was by comparing their retention times with those in a mixed-standard (FAME mix, Supelco, USA). For each sample, relative FAMES composition was quantified and data were presented as percentage weight for FAMES composition.

A total of 18 sausages (three sausages for each batch x three batches x two replicates) were analyzed for different parameters. A completely randomized experimental design with three levels of emulsified canola oil (0%, 15%, and 30%) was used. Analyses of variance were performed with a confidence interval of 95%, followed by the *post-hoc* Tukey test to establish statistical differences between treatments using SPSS 17.0 (SPSS, 2008).

RESULTS AND DISCUSSION

Chemical compositions of the Italian-type salami are shown in table 1. Except for moisture content, both fat and protein contents in the salami were according to the limits established by Brazilian legislation (IN n° 22) (BRASIL, 2000). As canola oil was added in an emulsified form, significant increases ($P < 0.05$) in moisture contents were observed in both T1 and T2 compared with those in Control. Several studies have reported that the higher moisture content in meat products were due to decreased fat content and the presence of liquid fat in and on the surface of Italian-type salami made with emulsified canola oil (BLOUKAS et al., 1997; PELSNER et al., 2007). In addition, higher moisture content reported in Italian-type salami made with emulsified canola oil could come from water added in the emulsion. UTRILLA et al. (2014) also related higher moisture content of dry-ripened venison sausages with higher proportion of olive oil throughout ripening time; although, differences were not particularly marked. In processing of dry-fermented meat sausages, a_w is an important parameter once it sets water available for enzymatic and microbiological reactions. In salamis made with emulsified canola oil, BACKES et al. (2013) related a_w values between 0.86 - 0.88 after 28 days of ripening. In our study, moisture values and T1 and T2 were 2% higher than those established by Brazilian legislation (BRASIL, 2000) and are not able to produce significant changes on final quality of salamis. In addition, according to RICKE & KEETON (1997), meat products with moisture content up to 39% are considered shelf stable.

In contrast to moisture content, significant decreases ($P < 0.05$) in fat contents of the salami made with emulsified canola oil were observed compared with those in Control (Table 1). However, this effect was independent of the amount of emulsified canola oil added. Nevertheless, the fat content of Italian-type

salami agrees with the Brazilian legislation, with the fat content set at a maximum of 32% (BRASIL, 2000). Protein contents were not significantly different between Control and T1 and T2. However, protein contents of our salami were similar to those of fermented meat sausages with canola oil, as reported by PELSNER et al. (2007). The ash content of salami (T1) was significantly lower ($P < 0.05$) than that of salami (T2) and Control, and 7% was acceptable for fermented meat products.

Microbiological properties of Italian-type salami are shown in figure 1. LAB values increased from 6.00log colony-forming unit (CFU) g^{-1} at day 0 to 8.00log CFU g^{-1} at day 5 during the fermentation process. The rapid growth of lactic acid bacteria (LAB) is desirable in fermented meat products, such as Italian-type salami, to prevent the growth of pathogenic bacteria (RACCACH, 1992). Growth of LAB was observed during the fermentation process and remained stable until the end of the process (day 28). LAB is the dominant group of microorganisms in fermented meat sausages, and they multiply to exceed 8log CFU g^{-1} at the end of the process (BENITO et al., 2007). The count of total coliforms decreased during fermentation. At day 28, total coliforms were not detected in salami (T1 and T2), and 1.10log CFU g^{-1} was reported in Control. Furthermore, fecal coliforms, coagulase-positive staphylococci, and *Salmonella* were not detected in Italian-type salami with emulsified canola oil. Brazilian legislation (RDC n° 12) establishes maximum counts of 3log CFU g^{-1} for fecal coliforms, 3.7log CFU g^{-1} for coagulase-positive staphylococcus and absence of *Salmonella* in 25g of sample (BRASIL, 2001). In addition, our microbiological results are in agreement with those reported by BLOUKAS et al. (1997), MUGUERZA et al. (2001), and DEL NOBILE et al. (2009).

The fatty acid profile, SAFAs, MUFAs, PUFAs, and PUFA/SAFA ratio of Italian-type salami are shown in table 2. Canola oil mostly consists of

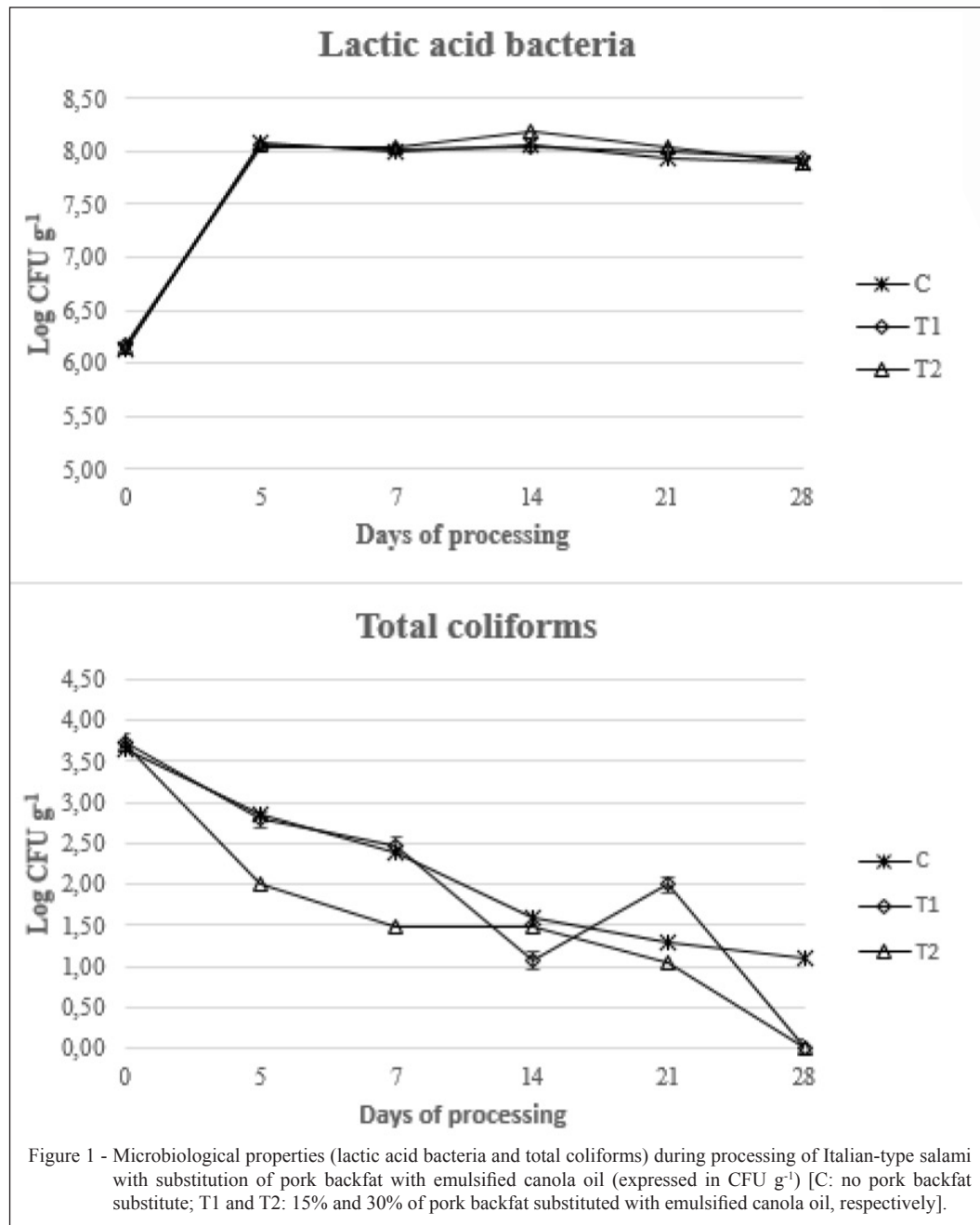
Table 1 - Effects of substituting pork backfat with emulsified canola oil on the chemical composition of Italian-type salami (expressed in $g\ 100g^{-1}$).

	-----Treatments-----			Legislation*
	C	T1	T2	
Moisture	34.56±0.62 ^b	36.94±0.58 ^a	37.03±0.23 ^a	Max. 35.00
Fat	28.48±0.51 ^b	24.06±0.81 ^a	25.54±0.82 ^a	Max. 32.00
Protein	28.36±0.42	28.06±0.56	28.87±0.72	Min. 25.00
Ash	7.42±0.10 ^a	7.17±0.06 ^b	7.50±0.08 ^a	-

^{a-b}Mean values in the same row not followed by a common letter differ significantly ($P < 0.05$);

C: no pork backfat replacement; T1 and T2: 15% and 30% of pork backfat substituted with emulsified canola oil, respectively;

*Values according to Brazilian legislation (BRASIL, 2000).



MUFA (oleic, C18:1n9) and PUFA (linoleic, C18:2n6) and a small amount of SAFAs, mainly consisting of palmitic (C16:0) and stearic (C18:0) acids. O'BRIEN (2008) reported that canola oil consisted of half the amount of SAFAs compared with soybean and corn oils, but it is rich in unsaturated fatty acids.

With respect to the fatty acid profile, significant reductions ($P < 0.05$) in myristic (C14:0), palmitic (C16:0), stearic (C18:0), and arachidic

(C20:0) fatty acids were observed in salami with canola oil (T1 and T2) compared with those in Control (Table 2). However, a significant reduction ($P < 0.05$) in saturated margaric (C17:0) acid was observed only with 30% substitution. Significant reductions of SAFAs levels, including C14:0 and C16:0, improved the nutritional value of our salami, and this was attributable to the lipid profile of canola oil. Similar findings in frankfurter sausages and hamburger with

Table 2 - Fatty acid content (expressed in % of total fatty acids methyl esters), saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), PUFA/SAFA and n6/n3 ratios in Italian-type salami with pork backfat substituted by emulsified canola oil.

	-----Treatments-----			
	C	T1	T2	Canola oil
C14:0	0.97 ± 0.08 ^a	0.73 ± 0.06 ^b	0.79 ± 0.05 ^b	0.05 ± 0.02
C16:0	27.27 ± 0.40 ^a	19.71 ± 0.74 ^b	19.86 ± 0.42 ^b	3.80 ± 0.43
C17:0	0.60 ± 0.03 ^a	0.53 ± 0.03 ^{ab}	0.47 ± 0.02 ^b	ND
C18:0	13.95 ± 0.25 ^a	10.79 ± 0.27 ^b	9.87 ± 0.45 ^c	1.80 ± 0.22
C20:0	0.74 ± 0.07 ^a	0.17 ± 0.01 ^b	0.20 ± 0.01 ^b	0.43 ± 0.09
C16:1n7	1.58 ± 0.03	1.36 ± 0.07	1.42 ± 0.05	0.16 ± 0.03
C17:1n7	0.41 ± 0.01 ^a	0.44 ± 0.03 ^a	0.38 ± 0.00 ^b	ND
C18:1n9	47.48 ± 0.50 ^b	49.69 ± 1.22 ^a	49.12 ± 0.92 ^a	51.41 ± 1.13
C20:1n9	0.15 ± 0.05 ^b	0.71 ± 0.05 ^a	0.65 ± 0.06 ^a	0.67 ± 0.34
C18:2n6	2.62 ± 0.29 ^b	11.06 ± 0.43 ^a	11.98 ± 0.95 ^a	32.91 ± 0.20
C18:3n3	0.12 ± 0.02 ^c	0.39 ± 0.04 ^b	0.59 ± 0.07 ^a	4.69 ± 0.27
C20:2n6	0.08 ± 0.03	0.08 ± 0.06	0.05 ± 0.04	0.34 ± 0.06
∑ SAFAs	43.53 ^a	31.93 ^b	31.19 ^b	6.08
∑ MUFAs	49.62 ^b	52.20 ^a	51.57 ^a	52.24
∑ PUFAs	2.82 ^b	11.53 ^a	12.62 ^a	37.94
PUFA/SAFA	0.06 ^b	0.36 ^a	0.40 ^a	6.24
n6/n3	22.50 ^b	28.56 ^a	20.38 ^c	7.08

^{a-c}Mean values in the same row not followed by a common letter differ significantly ($P < 0.05$);

C: no pork backfat substitute; T1 and T2: 15% and 30% of pork backfat substituted with emulsified canola oil, respectively.

ND: Not detected.

canola oil addition have been reported (CHOI et al., 2010; SELANI et al., 2016).

Although canola oil contained high levels of MUFAs, only gadoleic (C20:1n9) fatty acid was significantly increased in salami made with emulsified canola oil compared with that in Control (Table 2). However, MUFAs levels were significantly increased in salami with canola oil. Other studies have reported increases in MUFAs contents in fermented meat products with pork backfat substituted with olive oil (MUGUERZA et al., 2001; DEL NOBILE et al., 2009). In contrast, PELSER et al. (2007) reported a reduction in MUFAs contents in fermented meat sausages with linseed oil but an increase when canola oil was used.

As shown in table 2, PUFAs, linoleic (C18:2n6), and linolenic (C18:3n3) fatty acids were significantly increased in salami with emulsified canola oil compared with those in Control. In addition, the increase in C18:3n3 fatty acid was reflected by the amount of canola oil added. However, the low level of eicosadienoic (C20:2n6) fatty acid in canola oil did not change its level in the salami. Variations in the fatty acid profiles of Italian-type salami with emulsified canola oil are in agreement with other meat products containing vegetable oils (PELSER et

al., 2007; CHOI et al., 2010; ASUMING-BEDIAKO et al., 2014; BAEK et al., 2016; SELANI et al., 2016).

Substituting pork backfat with emulsified canola oil significantly reduced ($P < 0.05$) SAFAs levels and increased ($P < 0.05$) MUFAs and PUFAs levels in salami (T1 and T2) compared with those in Control. According to LI et al. (2015), PUFAs reduced cholesterol levels in the blood plasma and thus lowers blood pressure and prevents cardiac arrhythmias. The PUFA/SAFA ratio is one of the parameters currently used to assess the food nutritional quality with respect to lipid content (ANSORENA & ASTIASARÁN, 2004). The ratio should be higher than 0.4 to reduce the negative effects of SAFAs on plasma low-density lipoprotein (LDL) cholesterol levels (ENSER et al., 2000). As shown in table 2, significant increases in PUFA/SAFA ratios are observed in salami with canola oil (T1 and T2) compared with those in Control. High n6/n3 ratios are associated with some health problems, including cardiovascular and inflammatory diseases. In counterpart, low n6/n3 ratios may exert suppressive effects (SIMOPOULOS, 2002). According to SALCEDO-SANDOVAL et al. (2014), for prevention of cardiovascular disease, the recommendation is to reduce the value to less than 4. However, some meats naturally have the n6/n3 ratio

higher than this value (WOOD et al., 2004). According to table 2, the replacement of pork backfat by emulsified canola oil (T2) was not enough to reduce n6/n3 ratio of meats to the recommended level, but the incorporation of 30% of emulsified canola oil (T2) was able (P<0.05) to reduce the ratio when compared to control (C) treatment. Therefore, substitution of pork backfat with emulsified canola oil improves the nutritional quality of Italian-type salami.

CONCLUSION

Substituting 15% and 30% of pork backfat with emulsified canola oil decreased the fat content and increased the moisture content of Italian-type salami. With the exception of moisture content, fat and protein contents were in agreement with Brazilian legislation. Addition of emulsified canola oil in Italian-type salami did not affect the expected growth of LAB, and pathogenic bacteria such as fecal coliforms, coagulase-positive staphylococci, and *Salmonella* were not detected during fermentation. Moreover, emulsified canola oil improves the fatty acid profile of Italian-type salami by decreasing SAFAs contents and increasing MUFAs and PUFAs contents; thus, canola oil increases the PUFA/SAFA ratio.

Our results showed that partial substitution of animal fat with emulsified canola oil could be used to diversify meat products and improve the fatty acid profiles of Italian-type salami. In addition, our methodology can be applied to other vegetable oils such as flaxseed, corn, and sunflower in various meat products.

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